Product Description SALSA® MLPA® Probemix
P036-E3 Subtelomeres Mix 1

To be used with the MLPA General Protocol.

Version E3. For complete product history see page 11.

Catalogue numbers:
- **P036-025R**: SALSA MLPA probemix P036 Subtelomeres Mix 1, 25 reactions.
- **P036-050R**: SALSA MLPA probemix P036 Subtelomeres Mix 1, 50 reactions.
- **P036-100R**: SALSA MLPA probemix P036 Subtelomeres Mix 1, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman capillary sequencers, respectively (see [www.mlpa.com](http://www.mlpa.com)).

Certificate of Analysis: Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at [www.mlpa.com](http://www.mlpa.com).

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: [www.mlpa.com](http://www.mlpa.com). It is the responsibility of the user to be aware of the latest scientific knowledge of the application before drawing any conclusions from findings generated with this product.

**Intended use:** This SALSA MLPA probemix P036 Subtelomeres Mix 1 is an in vitro diagnostic (IVD)\(^1\) or research use only (RUO) assay to detect deletion(s) and/or duplication(s) in subtelomeric regions in human DNA derived from peripheral blood, buccal swab, (un)cultured amniotic fluid obtained in week 16 of the pregnancy or later and free from blood contamination, (un)cultured chorionic villi free from maternal contamination, fetal blood, or products of conception as a potential cause of developmental delay, dysmorphic features, other congenital abnormalities and/or pregnancy loss. Detected abnormalities should always be confirmed by a designated MLPA follow-up probemix or other technique. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

\(^1\)Please note that this probemix is for In Vitro Diagnostic use (IVD) in the countries specified at the end of this product description. In all other countries, the product is for Research Use Only (RUO).

**Clinical background:** Aberrant copy numbers of subtelomeric regions, e.g. due to an unbalanced translocation, are a frequent cause of developmental delay and congenital abnormalities. This P036-E3 Subtelomeres Mix 1 probemix for MLPA provides a faster, higher-throughput and more economical means than microarray analysis and FISH to identify individuals with a copy number change in one or more subtelomeric regions. MLPA cannot identify balanced rearrangements, however, and has a lower detection rate than microarray analysis. Detection rates depend strongly on the patient cohort tested. Examples are 5.9% (Ahn et al. 2007) when testing 455 patients or 3.9% (Stegmann et al. 2008) when testing a patient cohort in which normal G-banding analysis did not detect any abnormalities. Note that the results of Ahn et al. included some samples containing a genetic anomaly that had been inherited from an unaffected parent and which hence might have been a polymorphism without clinical significance. More information on [https://www.orpha.net](https://www.orpha.net) and [http://decipher.sanger.ac.uk/](http://decipher.sanger.ac.uk/). See below for several publications on probemix P036 Subtelomeres Mix 1.

**Probemix content:** This SALSA MLPA probemix P036 Subtelomeres Mix 1 contains 46 MLPA probes with amplification products between 130 and 483 nt: 2 probes for each chromosome. 41 probes are located in subtelomeric regions. No probes are present for the subtelomeric regions of the 5 acrocentric chromosomes (13, 14, 15, 21, 22). For these, an extra probe is included detecting the q arm, close to the centromere. The subtelomeric probes for the X and Y chromosome are identical as they detect sequences in the pseudoautosomal regions (PAR1 and PAR2) which are identical in chromosome X and Y. More information is present in Table 1 of this document.
This Probemix contains ten quality control fragments generating amplification products between 64 and 121 nt: four DNA Quantity Fragments (Q-fragments), two DNA Denaturation Fragments (D-fragments), one benchmark fragment, and one chromosome X and two chromosome Y-specific fragments (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mlpa.com.

<table>
<thead>
<tr>
<th>Length (nt)</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>64-70-76-82</td>
<td>Q-fragments (Only visible with &lt;100 ng sample DNA)</td>
</tr>
<tr>
<td>88-96</td>
<td>D-fragments (Low signal of 88 or 96 fragment indicates incomplete denaturation)</td>
</tr>
<tr>
<td>92</td>
<td>Benchmark fragment</td>
</tr>
<tr>
<td>100</td>
<td>X-fragment (X chromosome specific) (AMOT gene)</td>
</tr>
<tr>
<td>105</td>
<td>Y-fragment (Y chromosome specific) (UTY gene)</td>
</tr>
<tr>
<td>121</td>
<td>Y-fragment: Specific for the Y chromosome (ZFY gene)</td>
</tr>
</tbody>
</table>

**MLPA technique:** The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.com).

**MLPA technique validation:** Internal validation of the MLPA technique using 16 DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation <0.10 for all probes over the experiment.

**Required specimens:** Extracted DNA from peripheral blood, buccal swab, (un)cultured amniotic fluid obtained in week 16 of the pregnancy or later and free from blood contamination, (un)cultured chorionic villi free from maternal contamination, or fetal blood, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

**Reference samples:** All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from unrelated individuals without developmental delay or congenital abnormalities. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

**Positive control DNA samples:** MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. The Coriell Institute (https://catalog.coriell.org) and DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. Sample ID number NA06047 (heterozygous 17p deletion (5.6 Mb) including the RPH3AL gene) from the Coriell Institute have been tested at MRC-Holland and can be used as a positive control sample. The quality of cell lines can change, therefore samples should be validated before use.

**Performance characteristics:** The exact performance characteristics cannot easily be determined due to the difficulty in obtaining sufficient DNA samples containing deletions or duplications for all probes. Ahn et al. (2007) determined 95% confidence intervals for positive and negative predictive accuracies to be 0.951-0.996 and 0.9996-1 respectively in their laboratory. In general, each positive result needs to be confirmed and further studied.

Analytical performance can be compromised by: SNPs or other polymorphisms (e.g. indels) in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.
**Data analysis:** Coffalyser.Net software must be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mlpa.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

**Interpretation of results:** The expected allele copy number for all probes, including the probes for the pseudoautosomal regions of the sex chromosomes, are 2 (normal), 1 (heterozygous deletion) or 3 (heterozygous duplication).

The standard deviation of all probes in the reference samples should be <0.10 and the dosage quotient (DQ) of the reference probes in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results for autosomal or pseudo-autosomal chromosomes:

<table>
<thead>
<tr>
<th>Copy Number status</th>
<th>Dosage quotient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.80 &lt; DQ &lt; 1.20</td>
</tr>
<tr>
<td>homozygous deletion</td>
<td>DQ = 0</td>
</tr>
<tr>
<td>heterozygous deletion</td>
<td>0.40 &lt; DQ &lt; 0.65</td>
</tr>
<tr>
<td>heterozygous duplication</td>
<td>1.30 &lt; DQ &lt; 1.65</td>
</tr>
<tr>
<td>heterozygous triplication/ homozygous duplication</td>
<td>1.75 &lt; DQ &lt; 2.15</td>
</tr>
<tr>
<td>ambiguous copy number</td>
<td>all other values</td>
</tr>
</tbody>
</table>

- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.

- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.

**Notes P036 results:**

- Developmental delay or other abnormalities due to subtelomeric deletions or duplications is in most cases an autosomal dominant disorder. Rearrangement of a single chromosome copy can be pathogenic.
- Deletions can result in haploinsufficiency of one or more genes. Duplications can lead to distortion of gene expression or can result in haploinsufficiency of genes that are disrupted by the duplication breakpoints. Analysis of parental samples may be necessary for correct interpretation of results.
- Not all subtelomeric abnormalities detected by MLPA are pathogenic. In case of positive results, it is therefore strongly recommended to also test the parents. For each subtelomeric region, the copy number of only a single small (70 nt) sequence is determined. These sequences have been selected on the basis of their proximity to the telomere and are usually located in genes that have NOT been directly implicated in developmental delay or congenital abnormalities. For many chromosomes, it is not known what minimum size is required for a subtelomeric deletion/duplication to indeed result in developmental delay or congenital abnormalities. Finally, the frequency of benign copy number changes (CNVs) is higher in the subtelomeric regions (http://dgv.tcag.ca/dgv/app/home).
- In some instances, the nearest gene is mentioned in Table 1 as the gene that is detected by a particular probe.
Limitations of the procedure:

- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region do exist but remain undetected.
- For use on (un)cultured amniocytes, contamination of the sample with maternal DNA may lead to wrong conclusions.
- For use on (un)cultured chorionic villi, discrepancies in chromosomal patterns between DNA from chorionic villi and foetus have been described due to maternal contamination, postzygotic nondisjunction, postzygotic isochromosome formation, mosaic situations, and complications in DNA sampling in twin pregnancies (Van den Berg et al. 2006).
- Sequence changes (e.g. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.

Confirmation of results: Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polyorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the sample DNA. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Given the high chance of false positive/negative results, it is strongly recommended to use both MLPA probemix P036 and MLPA probemix P070 for initial testing of each DNA sample. All P070 probes differ from P036 probes. Copy number changes detected by P036 and P070 may still be without clinical significance. In case of positive results, it is therefore strongly recommended to also test the parents. In case P036 and P070 give conflicting results, follow-up studies are needed.

To confirm and delineate the extent of a subtelomeric deletion/duplication, MLPA follow-up probemixes containing 6-12 probes for a particular subtelomeric region are available. Confirmation may also be done by FISH, microarray or low coverage whole genome sequencing. Confirmation of results may be difficult: deletions/duplications can be interstitial and may not extend to the telomere. P036 and P070 probes may detect sequences which are located quite far from each other or from commercially available FISH probes.

Database of Genomic variants and Phenotype: http://decipher.sanger.ac.uk/. We strongly encourage users to deposit positive results in the Decipher database. Please report false positive results due to SNPs and other unusual results to MRC-Holland: info@mlpa.com.

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2 The finding of false negative results is not unlikely given that each chromosome arm is (usually) covered by a single probe only; whereas, false positive results may be due to polymorphisms as well as normal variation in subtelomeric regions.
Complete probe sequences are available on request:

result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

Complete probe sequences are available on request: info@mlpa.com.

**Table 1. SALSA MLPA Probeset P036-E3 Subtelomeres Mix 1**

<table>
<thead>
<tr>
<th>Length (nt)</th>
<th>Chromosomal position</th>
<th>Gene detected</th>
<th>SALSA MLPA probe</th>
<th>MapView build 36 position</th>
</tr>
</thead>
<tbody>
<tr>
<td>64-121</td>
<td>Control fragments – see table in probemix content section for more information</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>130 «</td>
<td>1p</td>
<td>TNFRSF4</td>
<td>02269-L01761</td>
<td>01-001.14</td>
</tr>
<tr>
<td>136</td>
<td>2p</td>
<td>ACPI</td>
<td>02274-L08758</td>
<td>02-000.25</td>
</tr>
<tr>
<td>142</td>
<td>3p</td>
<td>CHL1</td>
<td>01721-L01329</td>
<td>03-000.34</td>
</tr>
<tr>
<td>151</td>
<td>4p</td>
<td>PIKG (FLJ20265)</td>
<td>02005-L02047</td>
<td>04-000.50</td>
</tr>
<tr>
<td>158</td>
<td>5p</td>
<td>PDCD6</td>
<td>01723-L01327</td>
<td>05-000.37</td>
</tr>
<tr>
<td>166</td>
<td>6p</td>
<td>IRF4</td>
<td>01724-L02048</td>
<td>06-000.34</td>
</tr>
<tr>
<td>172</td>
<td>7p</td>
<td>ADAP1 (CENTAI)</td>
<td>02275-L02049</td>
<td>07-000.93</td>
</tr>
<tr>
<td>179</td>
<td>8p</td>
<td>FBXO25</td>
<td>02397-L01845</td>
<td>08-000.40</td>
</tr>
<tr>
<td>186</td>
<td>9p</td>
<td>DMRT1</td>
<td>01727-L02050</td>
<td>09-000.84</td>
</tr>
<tr>
<td>193</td>
<td>10p</td>
<td>DIP2C (KIAA0934)</td>
<td>02277-L01768</td>
<td>10-000.48</td>
</tr>
<tr>
<td>202</td>
<td>11p</td>
<td>RIC8A (RIC-8)</td>
<td>03315-L02733</td>
<td>11-000.20</td>
</tr>
<tr>
<td>208 «</td>
<td>12p</td>
<td>SLCGA12</td>
<td>02276-L01767</td>
<td>12-000.17</td>
</tr>
<tr>
<td>219 +</td>
<td>13q-cen</td>
<td>PSCP1</td>
<td>02399-L01847</td>
<td>13-019.24 (Acrocentric chromosome)</td>
</tr>
<tr>
<td>227 +</td>
<td>14q-cen</td>
<td>CCNB1</td>
<td>1</td>
<td>(HEI10)</td>
</tr>
<tr>
<td>235 + #</td>
<td>15q-cen</td>
<td>MKRN3</td>
<td>07291-L08858</td>
<td>15-021.36 (Acrocentric chromosome)</td>
</tr>
<tr>
<td>242</td>
<td>16p</td>
<td>POLR3K</td>
<td>01734-L01316</td>
<td>16-000.04</td>
</tr>
<tr>
<td>250</td>
<td>17p</td>
<td>RPH3AL</td>
<td>01735-L01315</td>
<td>17-000.17</td>
</tr>
<tr>
<td>258</td>
<td>18p</td>
<td>USP14</td>
<td>01736-L02051</td>
<td>18-000.19</td>
</tr>
<tr>
<td>265 «</td>
<td>19p</td>
<td>CDC34</td>
<td>01737-L01313</td>
<td>19-000.49</td>
</tr>
<tr>
<td>274 «</td>
<td>20p</td>
<td>SOX12</td>
<td>02396-L01844</td>
<td>20-000.26</td>
</tr>
<tr>
<td>283 +</td>
<td>21q-cen</td>
<td>RBM11</td>
<td>18223-L23852</td>
<td>21-014.51 (Acrocentric chromosome)</td>
</tr>
<tr>
<td>289 +</td>
<td>22q-cen</td>
<td>BID</td>
<td>01740-L01310</td>
<td>22-016.61 (Acrocentric chromosome)</td>
</tr>
<tr>
<td>296</td>
<td>Xp/Yp (PAR1)</td>
<td>SHOX</td>
<td>01148-L01331</td>
<td>X/Y-000.52 (PAR1 region)</td>
</tr>
</tbody>
</table>

- The 13, 14, 15, 21 & 22 q-cen probes target the q arm close to the centromere (acrocentric chromosomes).
- Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
- The specificity of this probe relies on a single nucleotide difference between a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

MRC-Holland MLPA

Product Description version E3-03; Issued 05 June 2018
Important notes on specific subtelomeres

IMPORTANT NOTE: several probes are located in regions that show copy number variation in healthy individuals! See http://dgv.tcag.ca/dgv/app/home. Therefore, we strongly recommend ALWAYS confirming results by a designated MLPA follow-up probemix or other suitable method.

Please inform us about any improvements or interesting findings: info@mlpa.com.

- **1p**: The **P070** probe for 1p detects a sequence that has been found to be duplicated in a normal individual. As deletions of this region may have consequences, we will not replace this probe.

- **3p**: The **P036/P070** probes for 3p (CHL1 gene) were found to be duplicated in healthy individuals. According to Dijkhuizen et al. (2006; *Am J Hum Genet*. 140A:2482-87), defects in the more centromeric CNTN4 and CRBN genes might be more important for the 3p syndrome than CHL1 loss.

- **12p**: The **P036/P070** probes detect sequences that were found to be duplicated in healthy individuals.

- **13q-cen**: This probe detects sequences on the q-arm, close to the centromere, as the p-arm of this chromosome does not contain well-characterised genes.

- **14q-cen**: This probe detects sequences on the q-arm close to the centromere, as the p-arm of this chromosome does not contain well-characterised genes.

- **15q-cen**: This probe detects sequences on the q-arm close to the centromere, as the p-arms do not contain well-characterised genes. The **P036** probe for 15q-cen detects the MKRN3 gene. This region is deleted in some Prader-Willi/Angelman syndrome patients. The PWS/AS critical region (SNRPN gene) is located at a distance of 1.3 Mb towards the q-telomere. We recommend the SALSA® MLPA® probemix ME028 Prader-Willi/Angelman for further characterisation of copy number changes of the MKRN3 region.

- **16p**: In several healthy individuals, a deletion of the P036 probe for 16p was detected, while the P070 probe showed normal results.

- **20p**: The **P036/P070** probes for 20p detect sequences that have been found to be duplicated in sporadic cases in a normal individual. As deletions of this region might have consequences, we will not replace this probe.

- **21q-cen**: This probe detects sequences on the q-arm close to the centromere, as the p-arm of this chromosome does not contain well-characterised genes.

- **22q-cen**: This probe detects sequences on the q-arm close to the centromere, as the p-arm of this chromosome does not contain well-characterised genes.

- **3q**: The **P036** probe for 3q (BDH1 gene) was found to be duplicated in a healthy parent by E. Reyniers, Antwerp (confirmed by FISH). The P070 probe for 3q, located 200 kb closer to the telomere, did not show this duplication. Apparently, copy number polymorphisms of the BDH1 gene region occur in healthy individuals. However, please note that a **3q29 microdeletion** syndrome (due to an interstitial deletion) has been described (Willatt et al. 2005; *J. Hum. Genet*. 77.154-160) and that the probe in the **P036** probemix detects a sequence within this commonly deleted region. The 3q probe in **P070** will not detect this microdeletion, as it detects a sequence located between the interstitial deletion and the telomere.

- **4q**: The 4q telomeric region is complicated and very few genes are present. The FRG1 gene is the only well-characterised gene in the terminal 2-3 Mb of 4q, but most FRG1-specific probes tested were found to be unreliable due to the presence of population-specific SNPs in FRG1. The 4q probe in P036-E is located at larger distance from the telomere; we do not expect this to cause many false-negative results as deletions of the telomeric 2-3Mb region of 4q do not seem to cause any phenotypic effects (Shao et al. 2008; *Am J Med Genet*. 146A:2242-51).

- **5q**: The **P036** probe for 5q was found to be influenced in one patient by a polymorphism in the first nucleotide after the ligation site. The **P036/P070** probes for 5q detect sequences that have been found to be duplicated in sporadic cases in a normal individual. As deletions of this region may have consequences, we will not replace this probe.

- **12q**: A ZNF10 duplication in a healthy parent with both the P036 and P070 have been reported.

- **19q**: The P036/P070 probes for 19q gave variable results in healthy controls.

- **22q**: The **P070** probe for 22q was found to be deleted in one healthy individual. The sequence detected by the **P036** probe for 22q has only one mismatch with a related sequence on chromosome 2. This is sufficient to generate a chr, 22q specific MLPA signal. However, when this region is to be sequenced, primer design is complicated as only 4 mismatches are present in the 350 nt region containing this probe sequence. The P188 probe set contains many probes close to the 22q13 telomere and can be used to confirm or further characterise 22q13 deletions.
- X, Y: The sex chromosome specific probes in P036/P070 detect sequences located on both X & Y chromosome close to the telomeres (PAR region) and will thus indicate the combined copy number of X and Y.

**Finding the genetic cause of congenital abnormalities with MLPA**

The number of genes whose defect can result in developmental delay is large. In some cases, particular phenotypic features suggest the involvement of a specific gene or chromosomal region. Numerous SALSA MLPA probemixes are available to find the cause of developmental delay with distinct syndromic features, such as RETT, Sotos and Prader-Willi/Angelman syndrome.

For individuals with (suspicion of) congenital abnormalities, the genetic cause is found only in a minority of cases. When using the P036 and P070 probemixes, we suggest following the strategy as outlined in Figure 1. These probemixes can be used for a broad screening of subtelomeric copy number variations. In case P036/P070 do detect any abnormalities, these findings should be confirmed by an MLPA probemix for a specific syndrome (see www.mlpa.com) or another technique (e.g. FISH, array-CGH). In certain cases, it is advisable to screen the DNA from the parents, if available.

In case no abnormality is found, SALSA MLPA probemix P245 Microdeletion Syndromes-1A can be used for screening for distinct microdeletion syndromes. If positive, these results can be further characterised by P371-P374 follow-up probemixes for specific syndromes. See P245 Microdeletion Syndromes-1A product description for details.

![Figure 1](image-url)

**Figure 1:** Flowchart suggesting how to test an individual with suspicion of developmental delay lacking clear syndromic features.

**SALSA MLPA probemix P036 Subtelomeres Mix 1 and P070 Subtelomeres Mix 2B**

When used correctly, screening with MLPA probemixes P036 and P070 will exclude the presence of abnormal copy numbers of subtelomeric regions in the majority of samples. MLPA will however not detect inversions or balanced translocations. Studies have indicated that 3-8% of all developmental delay cases are caused by aberrant copy numbers of subtelomeric regions (see references on p. 8-10).
MLPA® MRC-Holland

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SALSA MLPA probemixes P036 Subtelomeres Mix I and P070 Subtelomeres Mix 2B both contain one probe for each subtelomere and can be used to detect most deletions/duplications in the subtelomeric regions. It is strongly recommended to use both probemixes together to maximise the detection rate.

Most probes in the P036 and P070 probemixes target well-characterised genes at short distance of the telomere. An exception is made for the p-arms of chromosomes 13, 14, 15, 21 and 22 as these are covered by over 10 Mb of repeat sequences. Here, the “p” probes recognise one of the first genes on the q arm, close to centromere.

Interpretation of abnormal copy number findings in subtelomeric regions is complicated. Subtelomeric copy number changes can also occur in unaffected individuals and the effect of a deletion or duplication will depend on the genes involved. A considerable number of abnormalities detected by a single probe may not be the cause of any phenotypic effect but can be due to a rare polymorphism or a copy number change which is also present in one of the parents. For some chromosome arms, even large subtelomeric deletions or duplications (>1 Mb) can be inherited without a clear phenotypic effect; see the notes above. Not all abnormalities detected by the P036 and P070 probemixes are pathogenic. For all abnormalities detected, we strongly recommend testing parents to determine whether the copy number aberration in the patient is de novo. De novo deletions/duplications have a higher probability of being the cause of the developmental delay. The Database of Genomic Variants can be useful to verify whether copy number changes occur in normal individuals: http://dgv.tcag.ca/dgv/app/home.

**Related SALSA MLPA probemixes**

- **P070 Subtelomeres Mix 2B**
  Contains one probe for each of the 41 subtelomeric regions and 5 probes near the centromeric regions of the five acrocentric chromosomes. All probes differ from the P036 probes. We strongly recommend using both P036 and P070 on each sample.

- **P245 Microdeletion Syndromes-1A**
  Probes for 23 different microdeletion syndromes; can be used for primary screening of microdeletion syndromes.

- **P064 Microdeletion Syndromes-1B**
  Probes for 15 different microdeletion syndromes; can be used for primary screening of microdeletion syndromes.

- **P106 MRX**
  Probes for several genes involved in X-linked mental retardation.

- **P181 Centromere-1**
  Contains one probe for each of 41 different centromeric regions and an extra probe near the centromeric regions of each of the five acrocentric chromosomes, resulting in two probes for each chromosome.

- **P182 Centromere-2**
  Similar to P181 centromeres but all probes are different from the P181 probes.

- **P147 1p36**
  Probes for the 1p36 subtelomeric region.

More probemixes are available for specific syndromes, including RETT(-like) syndrome, DiGeorge, Prader-Willi/Angelman, Lissencephaly, Canavan, Williams syndrome and many more. See [www.mlpa.com](http://www.mlpa.com).

**References**

Selected publications using SALSA MLPA Probemix P036 Subtelomeres Mix 1

### P036 Product history

<table>
<thead>
<tr>
<th>Version</th>
<th>Modification</th>
</tr>
</thead>
<tbody>
<tr>
<td>E3</td>
<td>The 118 nt chromosome Y probe has been elongated to 121 nt.</td>
</tr>
<tr>
<td>E2</td>
<td>DNA denaturation control fragments at 88 and 96 nt replaced (QDX2).</td>
</tr>
<tr>
<td>E1</td>
<td>Probes for 1p and 4q replaced.</td>
</tr>
<tr>
<td>D2</td>
<td>Chromosome X and Y specific control fragments at 100 and 105 nt added.</td>
</tr>
<tr>
<td>D</td>
<td>Probes for 1p and 4q replaced. DNA denaturation control fragments at 88 and 96 nt added.</td>
</tr>
<tr>
<td>C</td>
<td>Probes for 1p, 15p near the centromere, 4q and 9q replaced.</td>
</tr>
<tr>
<td>B</td>
<td>Probes for 11p, 4q, 5q, 10q, 12q, 14q, 16q, 19q, 20q and 21q replaced.</td>
</tr>
<tr>
<td>A</td>
<td>First release.</td>
</tr>
</tbody>
</table>

### Implemented changes in the product description

**Version E3-03 – 05 June 2018 (04)**
- Product description adapted to a new template.
- Information regarding probemix P069 and the telomere follow-up probemixes has been removed.
- Warning added to table 1 for probes relying on its specificity on a single nucleotide difference between target and related gene or pseudogene.
- IVD use now includes Morocco.

**Version E3-02 – 14 December 2017 (03)**
- Products of conception has been added to the intended use.
- SALSA Artificial Duplication DNA SD025 is obsolete and information regarding SD025 has been removed.
- Note on page 3 added: In some instances the nearest gene is mentioned (table 1) as the gene that is detected by a particular probe.
- Table 1: 274 nt probe was marked as salt sensitive.
- Related Probemixes: P245 and P064 renamed to Microdeletion Syndromes 1A and 1B, respectively.
- References added to selected publications.

**Version E3-01 – 28 February 2017 (03)**
- Product description adapted to a new product version (version number added, small changes in Table 1).

**Version E2-01 – 07 October 2016 (03)**
- Product description restructured and adapted to a new template.
- Updated intended use and required specimens.

**Version 18 - 31 July 2014**
- Product description adapted to CE product template.

**Version 17 - 15 July 2014 (54)**
- Name of probemix changed from “Human Telomere-3” to “Subtelomeres Mix 1”.

### More information: www.mlpa.com; www.mlpa.eu

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**IVD** EUROPE*
**RUO** RUO

*comprising EU (candidate) member states and members of the European Free Trade Association (EFTA).
The product is for RUO in all other European countries.